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Evaluating acute physiological responses of porcine epidermis exposed to a pulsed 3.8 micron laser

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ABSTRACT

Five male Yorkshire pigs were exposed on their flank to 4 microsecond pulses of laser light from a Deuterium Fluoride 3.8 micron Laser at varying energies. A preliminary ED_{50} threshold for various skin reactions was determined for this laser exposure combination. The animal's skin was assessed for injury immediately, 1 hour, 24 hours and 72 hours post exposure. In general, energies below 3.2 J/cm^2 leave no lasting skin reaction. As energy increased above the threshold, erythema or skin reddening was easily visualized. High-energy pulses appear to produce a "rug burn" erythema without evidence of punctate hemorrage (bleeding) or coagulation. Laser exposure sites on the pigs were also biopsied to obtain histopathological results. These findings suggest that the principal effect of this type of in-vivo laser exposure is removal of the epithelium, while not damaging the papillary dermis or structures beneath the Basement Membrane Zone (BMZ).

Keywords: 3.8 micron lasers, ED_{50} threshold, Yorkshire pigs, epithelial changes, in-vivo laser exposure

INTRODUCTION

The purpose of this research is to determine skin reaction thresholds for use in evaluating laser safety standards. Recently, there has been great interest in the use of 3.8 micron lasers, and to date there have been no studies at this wavelength to ascertain the minimum irradiance necessary to cause a skin reaction. The current ANSI Standard Z136. 1-2000 (American National Safety Standard for Safe Use of Lasers) has the same maximum permissible exposure for both cornea and skin.[1] However the cornea and skin are fundamentally different, both in function and structure, thus resulting in different characteristics of interaction. The only injury threshold data for 3.8 micron lasers we were able to locate was that of Dunsky and Egbert.[2] It was felt that using this data for skin might result in overly restrictive exposure limits. However, the Dunsky data does provide a reasonable starting point for a dermal injury investigation. Our hypothesis is that the dermis is significantly less susceptible to damage than the cornea at 3.8 microns, and the current Maximum Permissible Exposure is overly conservative for skin exposures. The consequences of ocular injury are more severe than dermal injury but safety standards should still be based on the latest scientific information.

MATERIALS AND METHODS

A 3.8 micron laser producing 4 microsecond single pulses was used for all exposures. The spot size was square in shape, and approximately 4 cm^2. Exposures were performed on both flanks of the pigs using a grid pattern. This procedure was carried out in accordance with the Guide for the Care and Use of Laboratory Animals under a protocol approved by Uniformed Services University of the Health Sciences (USUHS) Institutional Animal Care and Use Committee (IACUC). Animal housing was in accordance with requirements listed in a research facility approved by the Council on Accreditation for Assessment and Accreditation of Laboratory Animal Care (AAALAC International).

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Five male Yorkshire pigs were used for this study (Archer Farms, Darlington, MD). The pigs were housed as described in Rico et al[3]. Each weighed 19 to 25 kg. Animals were maintained under anesthesia during exposures using isoflurane gas (Abbott Laboratories, North Chicago, IL) set at an infusion rate of 1 – 1.5% with oxygen flow at 22.0 ml/kg/min (10.0 ml/lb/min). Inductions were accomplished with a combination of injectable anesthetics (Ketamine 20 mg/kg and Xylazine 2 mg/kg). Buprenorphine 0.005 - 0.01 mg was administered to the subject animals as necessary for pain management. Follow-up pain control was accomplished as necessary. The animals were positioned based on skin exposure location (flank) and all monitoring of the test animals was conducted by direct observation and was non-invasive. Additionally, the level of anesthesia was determined using species appropriate reflex testing. Physiological parameters were monitored using a reflectance pulse oximeter, (Vet Ox SDI 4402, Heska Corp., Ft. Collins, CO) and temperature monitor, (Temp Plus 2080, IVAC Corp., San Diego, CA). Provision of a support system to maintain the test animals core body temperature within species-specific normal limits was accomplished throughout the entire procedure.

Under general anesthesia the hair on the flank of the pig was clipped using electric shears. A square, purple grid pattern using a medical skin marker (IZI Medical Products, Baltimore, MD) was made on the flank of each test animal to indicate the exposed areas. Each grid pattern was composed of two horizontal rows of 2 to 10 squares approximating 4 cm^2 each. Energy delivered varied between 0.01 J/cm^2 and 86.6 J/cm^2. Each exposure site was evaluated for gross morphologic changes at 0 hours, 1 hour, 24 hours and 72 hours. Laser exposed areas were assessed for any dermal reactions at each time interval. Skin biopsies were obtained for future analysis.

Evaluation of exposures was performed as described in Winston et al[4]. For this study, four different gross skin reaction levels were utilized to describe the data. The four visible reaction levels used in this study were (from lowest to highest energy):

1. Skin erythema - reddening of the skin due to dilatation of underlying blood vessels
2. First stage of epithelial change – we interpret this stage to be epithelial whitening
3. Second stage of epithelial change – whitening plus erythema deep to the first stage of changes
4. Final stage of epithelial change – diffuse area of profound erythema deep to the second stage without evidence of hemorrhage, while preserving the follicular remnants

Visual analysis of laser effects was accomplished by three graders in accordance with Rico et al. The reaction levels from 52 exposure sites were entered into a probit statistical analysis package to determine the estimated dose for 50% probability for the morphologic change observed (ED_{50}) (Easy Probit version 1.0 1998).[5]

RESULTS

The following acute photographs document the four reaction levels as defined in the materials and methods section. As we increased the energy from 0.01 J/cm^2 to 86.6 J/cm^2, we found observable epithelial changes. At increasing energy levels, deleterious changes to the epithelial layers were observed. Biopsies were taken for future analysis and confirmation of visual findings. Visual assessment of maximum energy exposure, however, appeared to confirm complete destruction of the epithelial layer. But, structures deep to the basement membrane zone appear to have been preserved because no hemorrhage was observed. Further, there appeared to be no evidence of escar (charred tissue) formation. No acute medical intervention was required for the test animals other than the maintenance of anesthesia.

In Figure 1, we observed erythema consistent with laser-induced trauma to the skin. The laser pattern as illustrated in Figure 2 highlights some inconsistency in beam profile as noted at the arrows. In addition, the epithelial whitening noted is consistent with our first stage of epithelial changes as noted above. Figure 3 points out the second stage of epithelial change, with additional areas of erythema (arrows 1, 2, 3) consistent with higher energies. In Figure 4 we observed the final stage of epithelial change (42.3 J/cm2). In Figure 4, one can discern the fine vascular tufts expected at the tops of the papillary dermis just below the epithelial basement membrane as well as the appearance of follicular remnants. There was no evidence of frank hemorrhage.

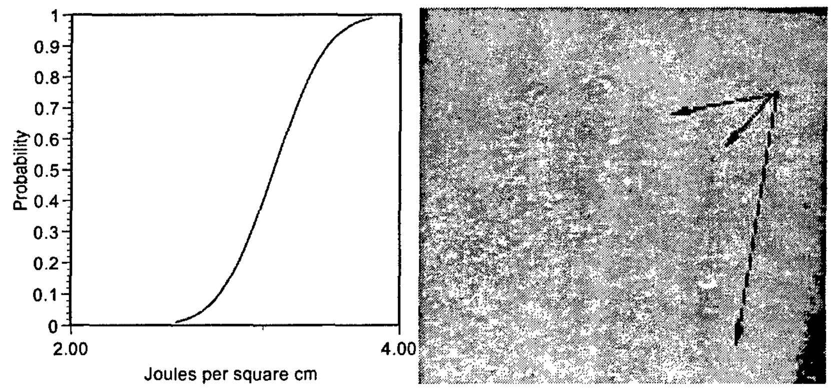

Figure 1. Probit curve for changes at 3.2 J/cm^2 – erythema (arrows)

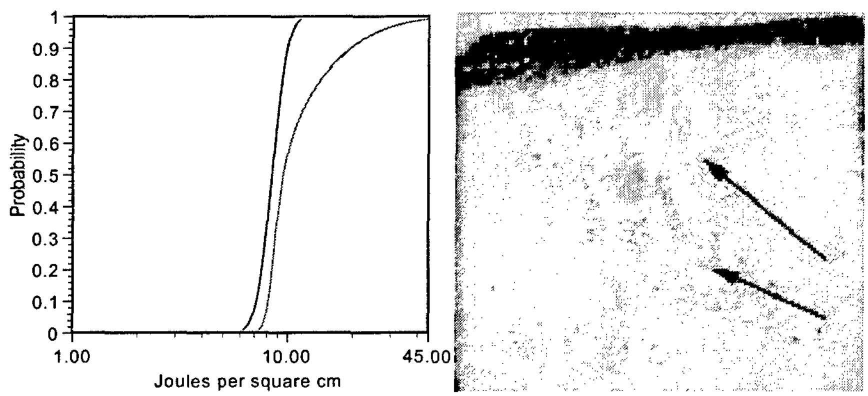

Figure 2. Probit curve for changes at 6.7 J/cm^2 – First stage of epithelial changes (arrows)

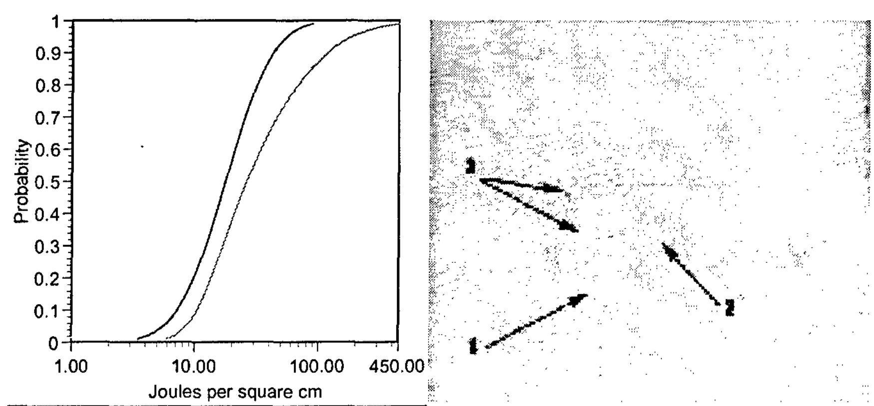

Figure 3. Probit curve for changes at 18 J/cm^2 - Second stage of epithelial changes, 26.4 J/cm^2 (arrows denote "hot spots")

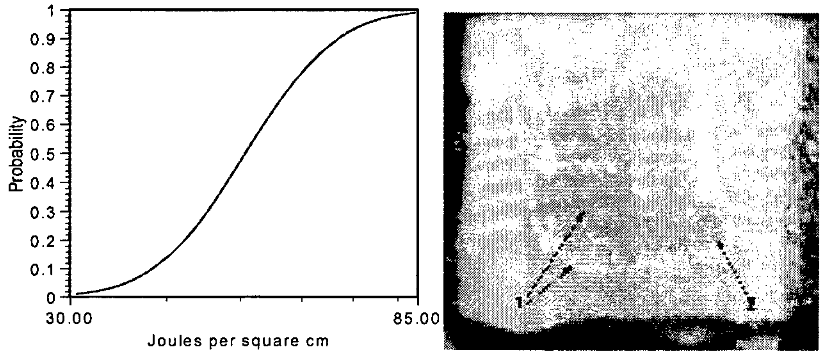

Figure 4. Probit curve for changes at 51 J/cm^2 - Final stage of epithelial changes, 42.3 J/cm^2 (arrows denote "hot spots")

DISCUSSION/CONCLUSION

It appeared to our observers that above 42 J/cm2 there was a threshold of damage beyond which further destruction of sub-basement membrane structures was not seen. We make this inference based on the fact that no evidence of hemorrhage or coagulation of vessels in the dermis (sub-BMZ) was noted. Follow-on histopathologic evaluation of these gross specimens will be necessary to confirm this inference. Also of note is the possibility that pigmentation may play a role in tissue response. Consideration by the team to adopt specialized staining to evaluate the microscopic change to melanocytes (specialized skin pigment production cells) has also been suggested. We also assume that the observed effect on the porcine tissue is a result of the laser energy. It is also possible that the energy from the laser heated retained water in the target tissue causing a "steam bubble." Mechanically, the steam bubble may, upon rupturing, blow the epithelium off the underlying dermis or may cause a mechanical compression wave that is propagated into deeper tissues. The observers felt that further evaluation of these possible mechanisms of damage should be evaluated.

Comparing the corneal data obtained by Dunsky et al (1×10^{-7} s exposure time vs. 4×10^{-6} s used in this study), we estimate there is a 10-fold difference between the energy required for gross tissue changes (erythema) in skin and Dunsky's energy limit for equivalent corneal reactions. Further experimentation and histologic review is planned to confirm this hypothesis. Current evidence suggests increasing the permissible exposure thresholds for skin effect from a 3.8 micron laser system by a factor of 10 relative to the Dunsky data set.

In our view, the diligent research into corneal safety standards accomplished by Dunsky, et al forms a responsible starting point for the assessment of a reasonable dermal exposure standard for the 3.8 micron laser. Implementing dermal standards will not only move forward medical treatments for laser exposures, but will also keep military personnel safe from laser-induced dermal injuries. Further, military personnel who understand the effects of dermal exposures are better equipped to defend themselves from future threats encountered by lasers on today's technologically advanced battlefield.

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